

IMPROVED APPARATUS AND METHOD FOR THE DETERMINATION OF THE VOLATILE OIL CONTENT OF SPICES*

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In 1928 Clevenger (1) introduced an apparatus for the laboratory estimation of the volatile oil content of plants and plant products. The method involved, which employed the principle of steam distillation of the relatively water-insoluble oil components, was intended to overcome the deficiencies (1) of the so-called "volatile ether-soluble extract" as a measure of essential oil content.

Previous attempts (2) had been made to establish an analytical method for the steam distillation of volatile oil, but the inconveniences of these methods and their lack of sufficient accuracy or precision precluded their acceptance. The Clevenger design was suitable for routine laboratory use and gave results considerably more reliable than previous steam distillation procedures: the quantity of oil obtained could be read directly in the trap and the uncontaminated oil was then available for further study of its chemical and physical properties.

Though still highly empirical as an estimate of the quality of the particular plant material, the Clevenger method as applied to spices was recognized as a distinct improvement over the "volatile ether extract" and a series of investigations was shortly undertaken by Clevenger and by this Association (3-6) to evaluate the trap design and details of the procedure. The method was adopted as tentative by this Association in 1934 (7) and has also appeared in the U. S. Pharmacopeia (8) and the National Formulary (9) for a number of years.

A second series of investigations was begun in 1949 by A. N. Carson, Associate Referee on Volatile Oil in Spices, for the purpose of modifying the tentative method to increase the reliability of the results. These studies (10) resulted in several changes, which were included in the modified procedure adopted as first action in *Official Methods of Analysis*, 7th Ed., 1950 (11). Changes in the original procedure already made or later suggested (12-14) by the Associate Referee include: Replacement of the cold finger by a West condenser, to prevent leakage of vapor (6, 10, 15); elimination of the practice of using ether to wash oil from the trap (10); use of ground glass connections (13); heating with a Glas-Col¹ heating mantle (13); and centrifugation of allspice oil to consolidate the lighter- and heavier-than-water fractions (14).

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¹ Mention of specific firms and products throughout this paper does not imply their endorsement by the Department of Agriculture to the possible detriment of similar firms and products not mentioned.

Modifications in the trap design itself were also considered, particularly for the determination of allspice (12, 13), but the disadvantages of one trap that had showed promise outweighed any gain from its use.

In 1954, the American Spice Trade Association began an analytical research program in collaboration with the U. S. Department of Agriculture, at the Eastern Regional Research Laboratory. One of the initial projects was a study of the volatile oil determination in spices. The particular objectives were increased precision, shorter analysis time, use of a smaller sample size when necessary, and over-all easier manipulation. In addition it was preferred that values obtained should be directly comparable to present standards as defined by various regulatory agencies; that is, it would not be desirable to substitute a totally different measurement for the "per cent volatile oil."

The plan of the investigation, then, was to modify the official method where necessary without discarding its basic principles. As shown below, the experimental work has resulted in both a modified apparatus and procedure; the over-all result seems largely to overcome the disadvantages of the present official method.

EXPERIMENTAL

The first problem considered was to decrease the length of time necessary for complete distillation of a sample. Simply increasing the heating mantle temperature would not provide a solution, because of the well-known tendency of spice materials to scorch and char. Likewise, use of salt solutions in the boiling flask to raise the distillation temperature would not only increase charring, but would introduce the possibility of changing the composition and amount of volatile oil. On the other hand, it was found that by agitating the contents of the flask, a higher heating mantle temperature could be used, resulting in a marked decrease in the length of time necessary for complete distillation. The method of choice for agitation is a magnetic stirrer, placed underneath the heating mantle. The effect of this stirring action is discussed below.

Study of the design of the Clevenger trap has led to a number of changes, which are incorporated in the trap shown in Figure 1 and Figure 2. This new trap differs in two respects from the Clevenger trap: (1) It consists of two parts, with the graduated portion being detachable; and (2) some of the dimensions of the trap have been changed. The advantages of this trap design are discussed below.

The procedure recommended for the use of this new trap is as follows:

Weigh accurately a sample (prepared as in *Official Methods of Analysis*, 8th Ed., 1955, 28.17) that will yield 0.25–1.5 ml oil (preferably more than 1 ml for greatest accuracy). Transfer to a 1 l flask in a heating mantle over a magnetic stirrer. Add about 500 ml H₂O and a Teflon-covered stirring bar with dimensions of at least 1½" × ½". Connect the trap and condenser and heat the flask, with stirring, setting the rheostat at about 80 v. or so that 3–4 ml H₂O will be distilled per minute. Arrange the condenser ("Ful-Jacket," preferably, with drip-tip), so that condensate runs down the wall of the trap, rather than directly on the surface of the liquid in the trap.

Distill until two consecutive readings taken at half-hour intervals show no change in oil content. Cool, centrifuge if necessary, and read the volume of collected oil, estimating to the nearest 0.005 ml.

Centrifugation.—Detach the graduated part, leaving the flexible tubing attached to this part. Place in a 50 ml centrifuge tube holder; balance against a second holder. Centrifuge 5–10 min., after gradually increasing the speed to 2,500 rpm. As a precaution against breakage, line the tube holder with heavy rubber sheeting.

Magnetic stirrer.—Most of the smaller-sized magnetic stirrers commercially available are suitable. These stirrers are specified as being able to “stir up to one liter of liquids with viscosities up to 60% glycerol solution.”

Heating mantle.—The mantle *without* an aluminum housing is preferable; however, the type with the metal housing can be used, but to obtain sufficient stirring it is usually necessary to decrease the distance between the top of the stirrer and the bottom of the flask, by flattening out the bottom of the metal housing.

RESULTS AND DISCUSSION

The effectiveness of agitation in reducing the time required for distillation is shown by the data in Table 1. The results were obtained by using

TABLE 1.—*Effect of stirring on distillation*

SAMPLE	TIME FOR COMPLETION ^a	
	WITHOUT STIRRING	WITH STIRRING
	<i>hours</i>	<i>hours</i>
Nutmeg	6	2
	6.5	1.5
	7	1.5
Sage	6–8	1–2
Celery seed	7	2–3
Clove	6–8	2–3

^a Heating mantle rheostat set at 55 v., without stirring; set at 80 v., with stirring.

the official procedure, with and without stirring. The criterion used for determination of complete distillation was no change in volume over two consecutive half-hour periods.

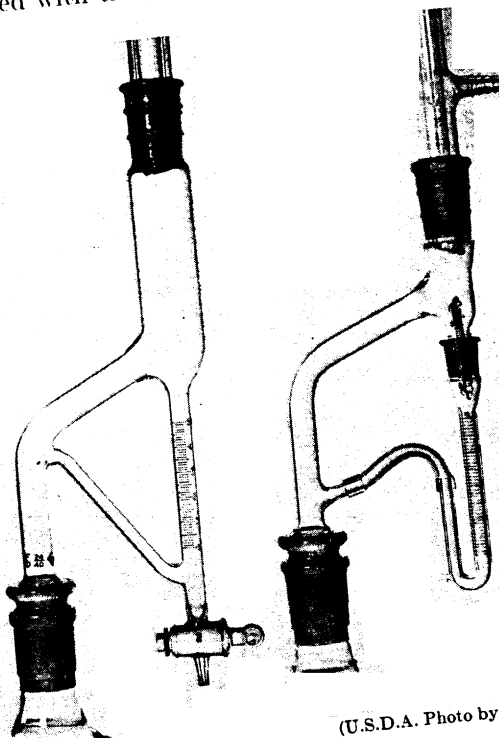
Stirring decreases the distillation time in three ways: First, the stirring action brings water and spice into more intimate contact; second, vapor is evolved uniformly—no superheating and local hot spots occur; third, and most important, the heating mantle can be kept at a higher temperature without significantly changing the pot temperature and without danger of scorching the sample. Use of the stirrer also eliminates the need for boiling chips and antifoaming agents.

The combined effects of stirring and use of the modified trap are shown in Table 2 and Table 3. The lighter-than-water trap was used for all spices except clove and allspice. For cassia samples, about 0.6 ml xylene² was

² Since the densities of cassia oils are very close to the density of water, a good separation of the two layers in the Clevenger trap usually does not take place; therefore, toluene is often added to the trap to dissolve the oil and keep the density of the organic layer sufficiently low so that it floats on the water layer. The authors prefer to use xylene, which is less volatile and less soluble in water than toluene is.

added to the graduated arm before distillation, and the volume was determined. The volume of cassia oil is found by subtracting the initial volume of xylene from the total volume at the end of the distillation and adding 0.040 ml to correct for the xylene dissolved and lost during the distillation.

Reference to Table 2 and Table 3 shows that the values closely approximate those obtained with the Clevenger trap and the official procedure,



(U.S.D.A. Photo by M.C. Audsley)

FIG. 1.—The Clevenger trap (left) and the new trap (right), for lighter-than-water oils.

even though smaller sample sizes and shorter distillation times are used. In a few cases the amount of separated oil is slightly larger than with the Clevenger trap, possibly because there is a smaller water layer in the graduated section, so that less oil is lost in saturating the water layer.

The advantages of the new trap, which was evolved after consideration of several designs, are as follows:

1. The calibrated portion of the "Precision-Bore" glass tubing is more accurate than the present official trap. It conforms to National Bureau of Standards specifications for a graduated 10 ml microburet.

2. The calibration unit (0.05 ml) is smaller than on the Clevenger trap (0.1 ml); yet adjacent graduations are farther apart than on the Clevenger trap, because the internal diameter of the new trap is much smaller. Much more accurate readings can be made and volumes can be determined to ± 0.005 ml. (Or instead, the size sample used can be much smaller without significant loss of precision or accuracy due to reading errors.)
3. Designing the trap in two parts results in several advantages. First,

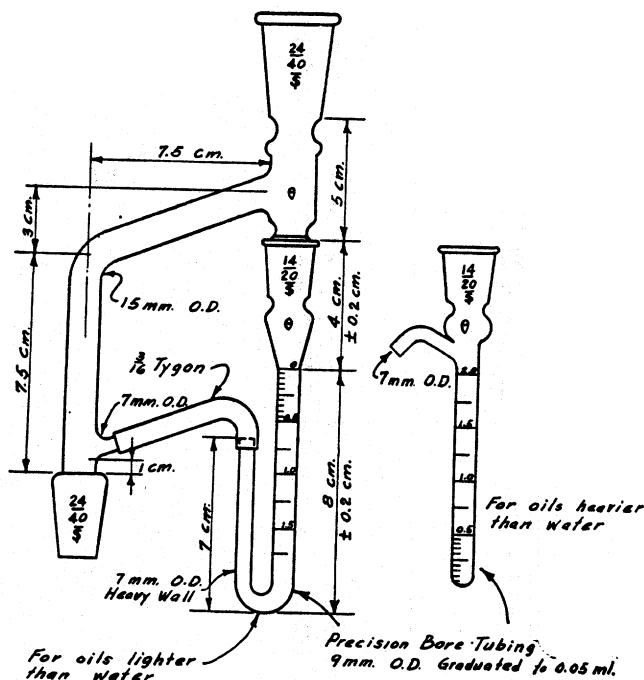


FIG. 2.—Diagram of the new trap.

the same set-up can be used for oils both heavier- and lighter-than-water. The proper graduated portion is attached to the one take-off (see Fig. 2); consequently, an entire set of both kinds of traps is not necessary. Second, it is easier to clean the two-section trap, especially the graduated portion, which must be extremely clean to prevent sticking or separating of oil globules. Third, where there is poor separation between the oil and water layers, or where droplets of one phase are dispersed in the other, the graduated portion can be detached and centrifuged, as described in the experimental section above. This produces a sharp meniscus, and both water and oil layers are free from cloudiness.

The dimensions of the trap (Fig. 2) are such that the graduated portion fits into a standard 50 ml centrifuge tube holder and can be centrifuged

TABLE 2.—*Steam-volatile oil, using the modified trap, lighter-than-water oils*

SAMPLE	APPROX. WEIGHT OF SAMPLE	VOLATILE OIL, (v/w), MODIFIED METHOD	VOLATILE OIL, (v/w), OFFICIAL METHOD
	<i>grams</i>	<i>per cent</i>	<i>per cent</i>
Nutmeg—1	50		4.4, 4.6
	25	4.76	
	20	4.71	
Nutmeg—2	50		6.6, 6.7
	25	6.72	
	25	6.69	
	10	6.77	
Nutmeg—3	50		5.0, 4.8
	40	5.08	4.9, 5.1
	25	5.09	
	25	4.98	
	20	4.99	
	10	4.90	
	5	4.95	
	5	4.98	
	2.5	4.96	
	2 ^a	4.96	
	1 ^a	4.93	
	1 ^a	4.89	
Fennel	30	1.51	
	25	1.48	
Celery Seed	50		2.6, 2.7
	30	2.65	
	25	2.62	
Coriander	45	0.33	
	40	0.34	
Cassia (Saigon)	50		2.0, 2.2
	25	2.28	
	25	2.30	
	20	2.31	
Cassia ^b (Batavia)	50		1.4, 1.3
	25	1.44	
	25	1.46	
Mace	20		11.0, 11.3
	15	11.07	
	10	11.04	
	10	11.05	

^a Micro-trap used; see text.^b Rheostat gradually increased to 80 v., after starting at 40 v.

in International Centrifuges, size 1 or 2, or the clinical model. If the clinical centrifuge is used, the standard rubber cushion may have to be replaced by a thinner one to insure adequate clearance.

4. As Fig. 1 shows, the dimensions of the assembly differ somewhat from the Clevenger trap. In particular, the long expanse of glass tubing immediately below the condenser joint has been eliminated. This length of tubing is a carry-over from the original cold finger design and now serves no purpose; its elimination results in greater cooling of condensate and leaves less glass area above the graduated portion to which oil can cling.

The smaller over-all dimensions of the trap also contribute to less ambient loss of oil—for example, the total volume of liquid in the trap and the return is less than half of that in the present official trap.

In addition to the trap shown in Figs. 1 and 2, several others are worthy of mention. One trap was made from 2 mm bore capillary tubing (in place of the Precision-Bore tubing) and 1 gram and 2 gram samples of nutmeg were distilled. Results were quite satisfactory and checked with larger weight samples (see Table 2). The use of this micro-trap, however, is of academic interest only, since such a small amount of material would not be a representative sample in most cases. Another variation in design was the substitution of $\frac{1}{8}$ 18/9 ball joints for the $\frac{1}{8}$ 14/20 cylindrical joints (Fig. 2). The ball joint works as well as the other joint, although the increased maneuverability is not needed, because the Tygon tubing provides sufficient flexibility for handling.

The results in Table 3 for allspice are not nearly as good as those for the other spices. There are two reasons for this: First, some small amount of oil remained on the surface of the water layer, even after extensive centrifugation; this variable quantity of oil (less dense than water) prevented an accurate reading of the total oil volume. Second, oil continued to distill from the sample for at least 48 hours. During the first two hours of distillation most of the oil was collected; later a slowly-distilling yellow fraction became noticeable. The rate of distillation of this yellow material, which was less dense than water, was about 0.01–0.05 ml per hour. Whether this particular allspice sample was atypical is not yet known.

As noted above, the trap, especially the graduated arm, must be thoroughly cleaned to prevent sticking and separation of globules. Cleaning by immersion of the graduated arm in hot sulfuric acid-nitric acid (1+1), followed by rinsing in water, usually is sufficient; detergent solutions, organic solvents, or strong sodium hydroxide solution are sometimes useful. With one sample (Indian celery seed) submitted to collaborative study, however, no amount of cleaning prevented separation of oil globules and their eventual siphoning back into the flask. Apparently, the volatile oil of this particular sample consisted almost entirely of sticky, very water-insoluble components which acted abnormally in the trap. Since

TABLE 3.—*Steam volatile oil, using the modified trap, heavier-than-water oils*

SAMPLE	APPROX. WEIGHT OF SAMPLE	VOLATILE OIL, (v/w), MODIFIED METHOD	VOLATILE OIL, (v/w), OFFICIAL METHOD
	<i>grams</i>	<i>per cent</i>	<i>per cent</i>
Allspice	50		3.6, 3.5
	25	3.75	
	25	3.50	
	20	3.27	
	20	3.58	
Clove	10	16.38	16.0, 15.6
	10	16.51	
	5	16.42	
	5	16.45	

portions of this same sample had been distilled without difficulty several months previously, and with higher results, it is presumed that there had occurred a loss of volatile oxygenated compounds which tended to wet glass and overcome the sticky action of the other components. The same difficulty occurred with this material when the Clevenger trap was used. This problem was solved by the addition of detergent, which effectively prevented any separation of oil globules. Several drops of a concentrated aqueous detergent solution were added at the top of the condenser after oil had begun to cling to the walls of the trap and to separate. As the detergent ran down the condenser wall, it mixed with condensing vapor and entered the trap. The oil layer almost immediately became homogeneous and acted normally. Sometimes this single addition of detergent prevented further separation throughout the remainder of the distillation, although at other times a further addition or two was necessary. No differences were found in yield, refractive index, or density, as compared to the occasional sample that would distill without separation (see Table 4). It is suggested, however, that the distillation be continued at least ten minutes after the final addition of detergent, in order to wash out practically all of the detergent added.

Of a large number of detergents tested, the anion-active "Nacconol NRSF" (an alkylbenzene sulfonate) worked best, although a non-ionic detergent is certainly preferable from the point of view of non-reactivity.

COLLABORATIVE WORK

Portions of the well-mixed ground spices (celery seed, nutmeg, and pepper) were sent to collaborators, along with instructions and sets of the new apparatus. The collaborative results are listed in Table 5.

Collaborator B had considerable difficulty with the celery seed sample, as discussed above, and his results are much lower than the others. (The use of detergent solution had not been investigated at the time of the

TABLE 4.—Physical constants of celery seed oil distilled in the presence of detergent

SAMPLE	REFRACTIVE INDEX, 20°C.	SPECIFIC GRAVITY AT 20°/4°C.
1 ^a	1.4816	0.846
2	1.4814	0.846
3	1.4821	0.847
4	1.4821	0.846

^a Detergent not added to this sample.

TABLE 5.—Collaborative results

LABORATORY	SAMPLE WEIGHT	DISTILLATION TIME	VOLATILE OIL (v/w)	\bar{x}	s	$\bar{\bar{x}}$	s_m^a
Indian Celery Seed							
	grams	hours	per cent				
A	20-50	3-4	2.06, 2.08, 2.06	2.07	0.012		
B	50	2.5	1.41, 1.49	1.45	0.057		
C	50	3-3.5	2.25, 2.14	2.19	0.078		
D	40	3	2.12, 2.21, 2.20, 2.12	2.16	0.052		
						2.13 ^b	0.062 or 2.92% ^b
E. Indian Nutmeg							
A	20-25	3	5.02, 5.06, 5.06	5.05	0.023		
B	20	4-4.5	5.02, 5.02, 5.02	5.02	0.000		
C	20	3-3.5	5.28, 5.25, 5.13, 5.13	5.20	0.079		
D	20-25	3	5.10, 5.12, 5.06, 5.07	5.09	0.027		
						5.09	0.079 or 1.54%
Lampong Black Pepper							
A	20	3	2.46, 2.48, 2.54, 2.58	2.52	0.055		
B	50	2.5-3	2.51, 2.53	2.52	0.014		
C	40-50	3	2.60, 2.56	2.58	0.028		
D	20-40	2.5	2.51, 2.50, 2.52, 2.52	2.51	0.010		
						2.53	0.032 or 1.26%

^a s_m = standard deviation of the collaborators' means.

^b Results of Collaborator B not included.

collaborative study.) This set of results was not included in the calculation of the standard deviation.

The closest comparison that can be made to other recent collaborative work is the data on nutmeg, for which results were reported in 1953 (12) and 1955 (14) by the Associate Referee. For a sample with an average volatile oil content of 7.5 per cent (8 collaborators), the interlaboratory standard deviation was 0.45, or 6.0 per cent (12). For a sample with an average volatile oil content of 7.7 per cent (5 collaborators), the interlaboratory standard deviation was 0.31 or 4.05 per cent (14). In the present study (4 collaborators) the interlaboratory standard deviation was 0.079 or 1.54 per cent.

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SUMMARY

Modifications were made in the design of the apparatus and in the procedure for determination of the volatile oil content of spices and other plant materials. These changes have resulted in shorter analysis time, easier manipulation, greater accuracy in reading the volume of oil, and increased precision. Details of the new method are presented and discussed, and the results of some collaborative work are given.

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